stability, conformation and binding affinity by equilibrium unfolding using steady state fluorescence and proteolytic digestion assay. These data show that imatinib binds to hFGF-1 and enhances its thermal stability and solvent accessibility. In addition, Biacore analysis was carried out to determine the binding affinity of imatinib to hFGF-1. ^{1}H-^{15}N HSQC NMR was also performed in order to determine exact binding sites and stoichiometry of binding between imatinib and hFGF-1.

273-Pos Board B53
A Shared Binding Site for Propofol and Thiopental in ELIC
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The intravenous general anesthetics propofol and thiopental target pentameric ligand-gated ion channels (pLGIC) and inhibit cation-conducting nAChRs. These drugs also inhibit ELIC, a prokaryotic pLGIC. However, the binding sites for these anesthetics are unknown for either nAChRs or ELIC. Here, using photoaffinity labeling, two-electrode voltage clamp electrophysiology and molecular docking, we identified a functionally relevant binding site for thiopental and propofol in ELIC. Molecular docking identified two binding pockets: an intrasubunit site near M265 of TM3, partially overlapped with the previously identified propofol binding site in GLIC; and an intersubunit site near W220 of TM1, which overlaps with the bromoform binding location identified previously. We generated two mutants, one targeted both predicted binding sites (W220F/W224F/M265C) and another targeted only the intrasubunit site (M265C). Functional measurements on Xenopus oocytes expressing the W220F/W224F/M265C mutant show a significant decrease of anesthetic inhibition, with a five-fold increase in the propofol IC50 and abolishment of thiopental inhibition. Interestingly, the M265C mutation alone could produce the same effect as the W220F/W224F/M265C mutant. Photoaffinity labeling experiments with a light-activated derivative of propofol (aziPm), in conjunction with mass spectrometry, confirmed the binding site at M265 for aziPm. Altogether, the results show that propofol and thiopental bind to a common functionally relevant site. This intrasubunit action site may also be shared by other intravenous anesthetics. Research supported by grants from the NIH.

274-Pos Board B54
Crystal View of Anesthetics and Alcohols Bound in the Pore of ELIC
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Cys-loop receptors, including the acetylcholine, glycine, 5-HT3 and GABA receptors, are molecular targets of general anesthetics and alcohols. Molecular mechanisms of anesthetics and alcohols interacting with Cys-loop receptors are still unclear. ELIC is a prokaryotic homolog of Cys-loop receptors and can be inhibited by general anesthetics and alcohols. Here, we report crystal structures (~3.1 Å) of ELIC bound with the volatile general anesthetic isoflurane, and bound with 2-bromoethanol. The crystal structures were obtained in the presence and absence of the agonist propylamine. Isoflurane was found inside the pore at two sites near T237(6′) and A244(13′), respectively, but 2-bromoethanol was only found near T237(6′). In addition, 2-bromoethanol also bound near Y102 and E150 in the extracellular domain. The presence of propylamine had no obvious effect on the binding sites for both isoflurane and 2-bromoethanol. This is the first time that an anesthetic or alcohol has been observed in the pore at an atomic resolution. The newly identified binding sites of isoflurane and 2-bromoethanol in ELIC are significantly different from previously reported anesthetic and alcohol binding sites. Neither isoflurane binding nor 2-bromoethanol binding introduced significant structural perturbation. The binding of isoflurane and 2-bromoethanol inside the pore suggests the possibility of channel occlusion as a mechanism for channel inhibition of Cys-loop receptors by general anesthetics and alcohols. Supported by grants from NIH.

275-Pos Board B55
Analysis of Antifolate Drugs with Disease Tissue Specificity
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Antifolates, analogues of the essential vitamin folic acid, are used in the clinic to treat cancers and inflammatory diseases. Antifolates are primarily transported into cells via the endogenously expressed reduced folate carrier (RFC). Conversely, our collaborators in Aleem Gangjee's group at Duquesne University have synthesized antifolates (AG antifolates) that transport poorly by the RFC, but are efficiently transported by the folate receptor (hFR). The GPI-anchored hFR is lowly expressed on the apical surface in a subset of normal epithelial lineages, but is highly expressed in many cancers of epithelial origin and on activated macrophages in inflammatory disease. Therefore, AG antifolate molecules have specificity for transport into disease cells over healthy cells. These newly developed AG antifolates cause cell death via inhibition of an enzyme involved in *de novo* purine synthesis, glycinamide ribonucleotide (GAR) transformylase.
We analyzed a series of AG antifolates using biophysical and biochemical techniques to understand both the specificity for transport by the folate receptor as well as the inhibition of the GAR transformylase in order to drive informed, hypothesis-based drug design. Our data, including pH-dependent binding profiles, enzyme inhibition data, and crystallographic models of protein in complex with AG molecules will be presented in the context of drug design and development.

276-Pos Board B56
PKA-Dependent Potentiation Mechanisms of Human CFTR Activity
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Both curcumin and VX-770 potentiate the channel activity of human CFTR (hCFTR) and two most common cystic fibrosis (CF) mutants G551D and F508delta in an ATP-independent but PKA-dependent manner. The underlying molecular mechanisms are unclear. Herein, HEK-293T cells cultured in a Fe^{3+}-containing medium were transiently transfected with hCFTR constructs and curcumin with well-known chemical nature was employed as a template to explore PKA-dependent potentiation mechanisms of hCFTR activity. The results showed that curcumin potentiation of Fe^{3+}-sensitive hCFTR activity was partially weakened by Fe^{3+}-insensitive mutations at the interface of the R domain and intracellular loop (ICL) 3 and completely suppressed by sufficient Fe^{3+}. Thus, release of the inhibitory Fe^{3+}-bound R domain from ICL3 by curcumin may be critical for curcumin potentiation. Further study indicated that curcumin potentiation was significantly prohibited by a missense alanine mutation of F157, Y161 or K166 from ICL1, or R1066, F1074 or F1078 from ICL4, or S795 or S813 from the R domain with or without the involvement of nucleotide-binding domain 2 (NBD2). More importantly, curcumin potentiation was also suppressed by the R811A/S813D or Y808A/S813D mutation and disulfide crosslinking of K162C to S795C enhanced channel opening. Therefore, the phosphorylated R domain may function as a length- and gating-regulatory cross-linker between two transmembrane domains (TMD1 and TMD2). Curcumin may potentiate hCFTR activity by stabilizing the stimulatory ICL1/ICL4-R interactions that promote channel opening by pulling all ICLs together and thus triggering a gating inward-to-outward reorientation of TMDs. Possible chemical interactions may involve cation-π interactions, π-π interactions and hydrogen bonding. Taken together, both release of the R domain from ICL3 and the stimulatory R-ICL1/ICL4 interactions may be necessary for PKA-dependent hCFTR activation and potentiation. These findings may help optimize the potentiators for treating those CF mutants with an ATP-dependent gating defect.

277-Pos Board B57
Kinase Structural Dynamics Enables Tight and Selective Binding of Inhibitors
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Protein kinases are obvious drug targets against cancer due to their central role in cellular regulation. With oncologic diseases being the second leading cause of death in the US kinases rapidly gain attention and are likely to become the number one drug target. Using NMR and fast kinetics, we establish a novel model that solves a longstanding question of high selectivity of clinically relevant drug Gleevec that effectively inhibits Abl tyrosine kinase while closely related Src family of kinases is affected much less. Our study of an entirely different family of Ser/Thr Aurora kinases and its specific inhibitors suggests that an energy landscape that provides tight affinity via an induced-fit and binding plasticity via a conformational selection mechanism is likely to be general for many inhibitors.

278-Pos Board B58
Glutathione Reductase of *Plasmodium Falciparum* as an Antimalarial Drug Target of Methylene Blue
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Plasmodium falciparum is the cause of human malaria and is one of two malaria parasites known to have drug resistance. Since there are no preventative